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Effects of hormone deficiency, androgen therapy and calcium supplementation on bone mineral density in female transsexuals

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Abstract

A total of 79 healthy female transsexuals, divided into four groups, were involved in this study. Group 1 comprised 15 pre-operated normal cycling females; Group 2, five pre-operated females who were on regular androgen therapy for 1–3 years; Group 3, 27 post-operated females who were on regular androgen therapy for 2–12 years; and Group 4, 32 post-operated females who either had stopped or were on irregular androgen therapy. A bone scan of the lumber spine, at positions L2–L4, was carried out for each subject. A blood sample was taken for measurement of plasma testosterone concentrations. Ten subjects from Group 3 had a repeat bone scan following 10–39 months of calcium supplement (625 mg daily as calcium carbonate); another 10 post-operated females of Group 3 had a repeat bone scan 6–59 months later; and five subjects from Group 4 had a repeat scan following resumption of regular androgen therapy for 17–27 months. The mean ± SE concentrations of testosterone of Groups 1–4 were, respectively, 0.58 ± 0.05, 10.1 ± 2.48, 7.7 ± 0.98 and 0.99 ± 0.14 ng/ml. Pre-operated females (Group 2) following 1–3 years of regular androgen therapy had significantly higher BMD and age-matched BMD than corresponding levels in pre-operated normal cycling females in Group 1. While the age-matched BMDs of post-operated females, who were on regular androgen therapy, were not significantly different, the mean BMD was significantly lower than corresponding values in the controls of Group 1. Post-operated females in Group 4 had significantly lower BMDs and age-matched BMDs as compared to corresponding values in controls of Group 1. The BMDs and age-matched BMDs of post-operated females, who were on regular androgen therapy, were significantly raised following daily calcium supplementation for durations ranging from 10–39 months. A repeat bone scan carried out following a lapse of 6–59 months did not reveal any significant change in the BMDs and age-matched BMDs of 10 post-operated females on regular androgen therapy. On the other hand, the BMDs and age-matched BMDs of post-operated females in Group 4 were significantly raised following the resumption of regular androgen therapy for 17–27 months. Results of the present study showed that ovariectomy and remaining in the hormone-deficient state for a sufficiently

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long duration was associated with a definite loss of bone mass. However, it was shown in this study that the resumption of regular androgen therapy for a sufficient duration could arrest this loss and, additionally, substantially increase the bone mass. Androgen appears to have a potentially greater impact on bone mass than oestrogen. Furthermore, calcium supplementation in a Singaporean population, which is accustomed to a low dietary calcium intake, can assist in the accretion of a higher bone mass in an adult population.

Keywords: Androgen therapy; Bone mineral density (BMD); Ovariectomised women; Calcium supplementation

1. Introduction

It is well established that oestrogens and androgens are involved in bone metabolism in humans [1–5]. Androgen and oestrogen receptors have been found in bone cells of both sexes [4,5]. Postmenopausal women or bilateral ovariectomised individuals, who remained in a prolonged state of oestrogen deficiency, may experience a progressive loss of bone mass with a resultant increase in the risk of osteoporosis. It has been shown that aging itself may also lead to a loss of bone mass in elderly people [6–9]. Hence, there are considerable difficulties in distinguishing between bone loss arising from hormone deficiency and aging in postmenopausal women.

The sex change surgery for female transsexuals includes bilateral ovariectomy, thereby creating a permanent state of oestrogen deficiency similar to that in postmenopausal women. In most post-operated transsexuals, however, this state of oestrogen deficiency occurred at a relatively younger age than those in most postmenopausal women. Therefore, using them as models in the present study had enabled us to evaluate the effects of hormone deficiency, androgen therapy and calcium supplementation on bone mineral density (BMD). Results from this study would provide some valuable insights into the relationship between hormone deficiency and bone loss and the efficacy of androgen therapy and calcium supplementation in maintaining bone mass in ovariectomised women.

2. Materials and methods

A total of 79 female transsexuals attending the Gender Identity Clinic at the National University Hospital were involved in this study. Apart from gender dysphoria, all subjects were healthy with no known illnesses. Besides the mandatory androgen therapy, all subjects involved in the present study were not on any other drugs or medications. Informed consent was obtained from each subject before the study commenced.

Subjects were divided into four groups. Group 1 comprised 15 pre-operated females (age, 20–32 years) who had regular menses. Group 2 comprised five pre-operated females (age, 22–37 years) who were on regular androgen therapy for 1–3 years. Group 3 consisted of 27 females (age, 25–39 years) who had completed their sex change operation from 0.5 to 10 years ago. They were on regular androgen therapy for a duration ranging from 2 to 12 years. Group 4 comprised 32 females post-operated for 0.5–13 years and had either stopped (0.5–8 years) or were on irregular hormone therapy (250 mg of Sustanon 3–4 monthly). A bone scan of the lumber spine, at positions L2–L4, was carried out for each subject. A blood sample was taken from most subjects on the day the bone scan was done.

Ten subjects from Group 3 had a repeat bone scan following 10–39 months of calcium supplement (625 mg daily as calcium carbonate) and these paired groups were designated as Groups 5A and 5B. Another 10 post-operated females on regular androgen therapy had a repeat bone scan 6–59 months later (Group 6A and 6B). Five subjects from Group 4 had a repeat scan following resumption of regular androgen therapy for 17–27 months (Group 7A and 7B).

2.1. Sex-change surgery for female transsexuals

The sex change surgery for female transsexuals involves multiple stages, comprising radical total

hysterectomy, reduction mastectomy, ovariectomy and the construction of a neophallus [10].

2.2. Androgen therapy

The regular androgen therapy regime for the population of female transsexuals in Singapore usually comprised 3–4 weekly intramuscular injections of 250 mg of Sustanon-250 (Organon) or a weekly injections of 1 ml of Depo-Testosterone (Upjohn, 100 mg of T-cyclopentylpropionate). Sustanon-250 is made up of a mixture of different testosterone esters; 30 mg of T-propionate, 60 mg of T-phenylpropionate, 60 mg of T-isocaproate and 100 mg of T-deconoate). The injections of androgen for all subjects were administered by nurses in our out-patient clinic.

2.3. Measurements of testosterone

Plasma concentrations of testosterone were measured by radioimmunoassay using the World Health Organisation Matched reagents and method previously reported [11]. The intra- and inter-assay coefficients of variation were less than 10%.

2.4. Bone scan of the spine

The bone scans were carried out in the Orthopaedic Diagnostic Centre, National University Hospital. The bone mineral density (BMD) was measured in the lumbar spine at positions L2–L4. The lumbar spine was scanned using dual energy X-ray absorptiometry (DEXA) with a Norland XR-26 Mark II bone densitometer. The bone mineral densities of the lumbar spine at positions L2–L4 were expressed as grams of calcium hydroxyapatite per cm^2 (BMD) as well as percentage (age-matched BMD) of the mean of an age-matched control population reported earlier [12]. DEXA measurements with the Norland XR-26 machine had been shown previously to give good precision (coefficient of variation of less than 1%), linearity and accuracy [13].

2.5. Statistical tests

The data were checked for normal distributions following which the one-way ANOVA test was used for statistical analyses of BMDs and age-matched BMDs of Groups 1–4. The student paired t-test was used to compare BMDs and age-matched BMDs of repeat scans in Groups 5A–7B, while unpaired t-test was used to compare means between the two groups. A Windows version of the SPSS statistical package was used for the tests done. Differences between groups were considered as statistically significant when the p-value was ≤ 0.05.

3. Results

Plasma testosterone concentrations reflect the status of androgen therapy of the four groups. In pre-operated, normal cycling females, the mean $\pm$ SE concentration of testosterone measured in the 15 subjects was 0.58 ± 0.05 ng/ml, while that for four subjects in Group 2 and 24 subjects in Group 3 were, respectively, 10.1 ± 2.48 ng/ml and 7.7 ± 0.98 ng/ml. Stopping or under treatment with androgen in 25 subjects from Group 4 was reflected by very low levels of testosterone, the mean $\pm$ SE was 0.99 ± 0.14 ng/ml. It must be noted that the blood samples were randomly collected in these subjects without reference to the time when the last i.m. injection of testosterone was administered. The wide scatter of testosterone noted in subjects in Groups 2–4 can be accounted for, in part, by the known uneven releases of testosterone from the depot preparation over the 3- to 4 week-intervals. It is also important to note that the current concentration of testosterone is not an index of its effect on bone mass. More importantly, it is the duration and dose of the historical exposure that matters, a point which was discussed in an earlier paper [14]. However, the low mean testosterone level in Group 4 concurred with the observation of lethargy, a more obvious clinical symptom of the lack of or under treatment with androgen [15], noted in many of these subjects.

The BMDs of the pre-operated normal cycling females in Group 1, whether expressed as g calcium hydroxyapatite/cm^2 (BMD) or as percent of age-matched control (age-matched BMD), were not significantly different from a normal population in Singapore [12]. Group 1 subjects, there-

Table 1
The BMDs and age-matched BMDs of all female transsexual groups

Group	*n*	Age-matched BMD ± SE	Mean ± SE BMD g hydroxyapatite/cm^2
Gp1 — Pre-operated and normal cycling	15	102 ± 8.2	1.154 ± 0.030
Gp 2 — Pre-operated and had 1–3 years of androgen therapy	5	117 ± 3.2[a]	1.228 ± 0.036[a]
Gp 3 — Post-operated and on regular androgen therapy	27	98 ± 1.5	1.061 ± 0.017[c]
Gp 4 — Post-operated and under-treated or had stopped therapy	32	81 ± 1.0[b]	0.873 ± 0.011[b]
Gp 5A — Subjects from Group 3	10	98 ± 1.1	1.037 ± 0.012
Gp 5B — Subjects of Gp 5A after 10–39 months of calcium supplement	10	104 ± 1.7[d]	1.119 ± 0.017[d]
Gp 6A — Subjects of Gp 3	10	97 ± 1.7	1.052 ± 0.018
Gp 6B — Subjects of Gp 6A 6–33 months later	10	98 ± 2.9	1.054 ± 0.024
Gp 7A — Subjects of Gp 4	5	78 ± 2.9	0.849 ± 0.028
Gp 7B — Subjects of Gp 7A after 17–27 months of regular androgen therapy	5	85 ± 3.8[e]	0.931 ± 0.042[e]

Age-matched BMDs are expressed as a percentage of the mean of a group of normal females matched for age.
[a]Significantly higher ($p < 0.05$, ANOVA) and [b]significantly lower ($p < 0.05$, ANOVA), respectively, than corresponding values in Gp 1.
[c]Significantly lower ($p < 0.05$, ANOVA) than corresponding value in Gp 1.
[d]Significantly higher ($p < 0.05$, paired t-test) than corresponding value in Gp 5A.
[e]Significantly higher ($p < 0.05$, paired t-test) than corresponding value in Gp 7A.

fore, served as controls for comparisons with the other groups.

Pre-operated females (Group 2) following 1–3 years of regular androgen therapy had significantly higher ($p < 0.05$, ANOVA) BMD and age-matched BMD, by 6.4%, and 15%, respectively, than corresponding levels in pre-operated and normal cycling females in Group 1 (Table 1).

The age-matched BMDs of post-operated females, who had been on regular androgen therapy (Group 3), were not significantly different from, but the mean BMD was significantly lower ($p < 0.05$ ANOVA) by 8%, compared to corresponding values in the controls of Group 1 (Table 1).

Post-operated females who either had stopped or were on irregular androgen therapy (Group 4) had significantly ($p < 0.05$, ANOVA) lower BMDs and age-matched BMDs compared to corresponding values in the controls in Group 1. The mean BMD and age-matched BMD in this group (Group 4) were significantly lower, by 25% and 21%, respectively, than corresponding values in the controls in Group 1 (Table 1).

Daily calcium supplementation for durations ranging from 10 to 39 months had significantly raised ($p < 0.05$, paired t-test), by an average of 7.9% and 6.0%, respectively, the BMDs and age-matched BMDs of posted-operated females who were on regular androgen therapy (Groups 5A and 5B, Table 1).

In 10 post-operated females on regular androgen therapy (Group 6A and 6B), the BMDs and age-matched BMDs did not change significantly ($p > 0.05$, paired t-test) when a repeat scan was carried out 6–59 months later (Table 1).

The BMDs and age-matched BMDs of post-operated females who had stopped or were on irregular androgen therapy were significantly raised ($p < 0.05$, paired t-test), by an average of 9.7% and 7.0%, respectively, following the resumption of regular androgen therapy for 17–27 months (Group 7A and 7B, Table 1).

4. Discussion

This study was undertaken to evaluate the impact of androgen therapy and ovariectomy on the bone mineral density in young and healthy women. Traditionally, bone mineral density has been used to predict long-term fracture risk [16]. Using BMD as the diagnostic criterion has its

limitations, especially when it does not give any indication of the important aspect of bone quality [1]. However, until such time when a better diagnostic criterion becomes available, which can take into account appropriate factors affecting bone mass and quality, BMD remains useful in the clinical management of bone disorders [1]. The BMDs or age-matched BMDs of pre-operated females in Group 1 were not significantly different from an age-matched population. In this respect, they were appropriate as controls for the present study.

Results of this study showed that the lumbar spine BMDs varied according to the state of hormone sufficiency or deficiency of the individual subjects. It is important to note that it is the historical exposure to androgen rather than the current plasma levels that is the determinant of the effect of androgen on bone mass. Interestingly, regular androgen treatment of pre-operated normal cycling women, for a mean duration of 1.4 years, as in transsexuals of Group 2, had significantly raised the BMDs when compared to control females in Group 1. This observation suggests that androgen may have a synergistic effect with oestrogen on bone metabolism inducing the increase in bone mass of the individuals. This suggestion is supported by earlier studies which showed that androgen given to postmenopausal women had led to increases in markers of bone formation [17–19]. It is noteworthy that after 1–3 years of androgen therapy, ovarian functions and hence, oestrogen production in female transsexuals, had been suppressed and all subjects become amenorrheic [20].

The positive effect of androgen on bone mass in a female system was further shown when BMDs in ovariectomised female transsexuals were maintained at normal levels when subjects were on regular androgen therapy, as was the case of subjects in Group 3 of the present study. This observation suggests that androgen can maintain bone mass in these ovariectomised women. The positive effect of testosterone on BMD was further shown when significant increases in BMDs were noted following the resumption of regular androgen therapy in those who either had stopped or were on irregular therapy (Group 5A and 5B). It addition, positive correlations between bone mass and androgen levels have been observed in premenopausal and postmenopausal women [21–23]. Thus the maintenance of bone mass and perhaps the attainment of higher BMDs might be due to the action of testosterone per se. This action is unlikely to be mediated solely by the conversion of testosterone to oestrogen. At the concentrations of testosterone attained in subjects under the current androgen therapy regime, we have shown earlier that the levels of oestradiol derived by peripheral conversion of androgen cannot match those present in normal cycling women [24]. Furthermore, although androgens and oestrogens may share the ability to decrease bone resorption, they appear to have different effects on bone formation [19,25,26]. However, because of the small number of subjects involved, the conclusion must be viewed with caution. The evaluation of the action of androgen per se on bone mass could be elucidated by the use of dihydrotestosterone, which is not aromatised to oestrogen, rather than testosterone preparations in future studies.

The results of the present study showed that either stopping or having irregular androgen therapy for prolonged periods of time had led to significant reductions of BMDs and age-matched BMDs in a group of post-operated females (Group 4) by an average of 25% and 21%, respectively, when compared to corresponding values in pre-operated and normal cycling females in Group 1. This observation shows that a hormone deficient state of sufficiently long duration can lead to a significant reduction of bone mass. The loss of bone mass observed in the group of females in the present study is unlikely to be age-related as such a reduction in BMD was seen in subjects with age ranging from 23 to 48 years old and as well as when BMDs were expressed as a percentage of an age-matched group. This conclusion, however, is only applicable to bone mass as reflected in the lumbar spine at positions L2–L4 and any extrapolation of the conclusion to other skeletal parts must be viewed with caution. More interestingly, it was shown that the decline in bone mass in such a group of individuals could be arrested and bone mass increased by the resumption of adequate androgen therapy.

Daily supplementation of 625 mg of mineral calcium in the form of calcium carbonate tablets in post-operated females on regular androgen therapy (Groups 5A and 5B) for 10–39 months, had significantly increased their BMDs and age-matched BMDs by an average of 7.9% and 7.0%, respectively. This implies that the increased availability of calcium in a group of females on regular androgen therapy could lead to the accretion of a higher bone mass.

Calcium supplementation and dietary calcium intake are controversial issues as regards their effects on bone mass [27–30]. Several studies have indicated that the increased availability of calcium can positively influence the peak bone mass [31,32]. A more recent study has shown that calcium supplementation had retarded lumbar bone mineral loss in perimenopausal women by decreasing the bone turnover [33]. The subjects in this study, however, were aged between 45 and 55 years and their dietary calcium intake was, on average, about 1150 mg Ca^{++}/day, which is considerably higher than that for the Singaporean population. Many of the earlier studies on the effects of calcium supplementation on bone mass were carried out in Caucasian populations, most having sufficient dietary intake of calcium. Several studies involving Asian populations were carried out in elderly and postmenopausal subjects and one in adolescents [34–37]; but none had examined the effect of calcium supplementation on peak bone mass. It is of upmost importance that appropriate studies be carried out in Asian populations, as factors, especially diet and lifestyle, are very different from those in developed countries. The average Singaporean diet contains less than 1200 mg of mineral calcium, the recommended daily allowance for Americans [38]. According to the Ministry of Health survey [39], the average dietary intake of mineral calcium for Chinese, which represented 95% of cases in the present study, is about 400 mg. Hence, daily calcium supplementation may be an important factor influencing the status of bone mass in the population.

The bone density of those who manifested osteoporotic fractures depends on both the rate of loss and the initial bone mass. Hence, it has been suggested that attention in osteoporosis research should be placed on skeletal growth in the first 20 years of life and to demonstrate the importance of mineral accrual (and factors influencing this accrual) in determining bone density in adulthood and old age [40]. Therefore, the demonstration that calcium supplementation can help increase bone mass in a group of adult Singaporean, who like many in other Asian populations, are on low dietary calcium intake, suggests that public health measures could be established to help the general population optimise their mineral accrual in the early part of life. Such a strategy may be more important than health care measures introduced closer to the age at which fractures and other problems associated with reduced bone mass occur.

In summary, the results of the present study showed that ovariectomy and remaining in a hormone-deficient state for a sufficiently long duration are associated with a definite loss of bone mass. In ovariectomised females, who had stopped their androgen therapy and had significant bone loss, the resumption of regular androgen therapy over a sufficiently long duration could arrest the decline and substantially restore the bone mass. Androgen appears to have a potentially greater impact on bone mass than oestrogen. In addition, calcium supplementation in a Singaporean population accustomed to a low dietary calcium intake, can improve bone mass in an adult population.

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